

THE SUSCEPTIBILITY AND RESISTANCE OF *BULINUS (PHYSOPSIS) GLOBOSUS* AND *BULINUS (BULINUS) TRUNCATUS ROHLFSI* TO TWO STRAINS OF *SCHISTOSOMA HAEMATOBIMUM* IN GHANA

FERGUS S. McCULLOUGH, M.Sc., Ph.D.

WHO Regional Office for Africa, Brazzaville, French Equatorial Africa
Formerly, Medical Biologist, Ministry of Health, Ghana

SYNOPSIS

The author presents the results of some laboratory experiments carried out to determine the susceptibility of the snail vectors of bilharziasis in Ghana to local strains of *Schistosoma haematobium*, isolated from children living in two districts near Accra: the Ke district, where *Bulinus (Bulinus) truncatus rohlfsi* is the important snail vector, and the Pokoasi district, where *Bulinus (Physopsis) globosus* is the exclusive intermediate host.

Each strain was found to be virtually specific in its ability to develop in one or other of the vector species. The "Ke strain" developed readily in *B. (B.) truncatus rohlfsi* but not in *B. (P.) globosus*, while the "Pokoasi strain" developed readily in the latter snail but not in the former.

The author points out that these findings provide a possible explanation for some of the conflicting observations that have been reported by workers in other African territories, and may have an important bearing on the epidemiology of urinary bilharziasis in Africa as a whole. He stresses the need for further studies on the relationship between bulinid snails and schistosome strains in Africa.

When considering the epidemiology of schistosome infections, special attention must be paid to the vector-parasite relationship both under controlled conditions in the laboratory and under natural conditions in the field. In Ghana (then Gold Coast), between 1952 and 1955, observations were made on the prevalence and epidemiology of human bilharziasis and on the distribution and ecology of the potential snail hosts (McCullough, 1956a). Furthermore, as urinary bilharziasis is far more widespread than *Schistosoma mansoni* infection, greater emphasis was placed on the relationship between *S. haematobium* and its potential bulinid vectors.

The most important vector of *S. haematobium* in Ghana is *Bulinus* (*Physopsis*) *globosus* (Morelet),¹ though in one area—the Ke district—*Bulinus* (*Bulinus*) *truncatus rohlfsi* (Clessin) is solely responsible for the transmission of the parasites (McCullough, 1956b). *Bulinus* (*B.*) *forskalii* (Ehrenberg), on the other hand, though widely distributed, appears to be refractory to local *S. haematobium* infection (McCullough, 1955a, 1955b). Thus in Ghana there are at least two quite distinct species, belonging to different subgenera, which can readily transmit *S. haematobium*. As each of these species occurs to the exclusion of the other in districts near the laboratory headquarters at Accra, this fortuitous circumstance seemed especially fortunate from the viewpoint of implementing the vector-parasite relationship studies described in the present paper.

Much information has accumulated on the degree of susceptibility or resistance of bulinid snails to *S. haematobium* infection. In this connexion Gismann (1954) and Kuntz (1955) are among those who have reviewed much of the literature. There is little doubt that our knowledge of the factors involved is still fragmentary, though, evidently, the explanation must lie either in the snails or in the parasites or in both. It seems, however, that the explanation of vector-parasite compatibility, or the lack of it, has pointed more strongly towards the snails than towards the parasites. This is in large measure due to the confusion that has long been apparent in the taxonomy of the African snail hosts. On the other hand, the role assumed by the parasites, *vis à vis* compatibility or otherwise, has scarcely been recognized owing to the complacent, if unjustified, acceptance of the systematics of the African *Schistosoma* species. The results of the present preliminary observations at Accra indicate that local strains of *S. haematobium* exist and that the degree of vector-parasite compatibility is dependent as much on them as on the bulinid snail hosts. It must be stressed, however, that it was not possible to pursue our investigations to the limits which seemed desirable and that the results are now described primarily as an incentive for further studies by other workers.

Experimental Procedure and Results

At Accra two local strains of *S. haematobium* were used for the purpose of the present experiments. One strain was obtained from children living at Kpotame near the Ke lagoon (McCullough, 1956b), where *B. (B.) truncatus rohlfsi* occurs and where *B. (P.) globosus* is apparently absent. For convenience this strain may be called the Ke strain of *S. haematobium*. The other strain—the Pokoasi strain—was obtained from children living at Pokoasi, where *B. (P.) globosus* occurs and *B. (B.) truncatus rohlfsi* is

¹ In my earlier papers this species was named *Physopsis africana* Krauss, 1848. The nomenclature of the snails given in the present paper follows that proposed by Mandahl-Barth (1957).

not found. Both in the Ke district and at Pokoasi, *B. (B.) forskalii* was found, but since there is as yet no direct evidence that the snails of this species can transmit *S. haematobium* in Ghana, it seems justifiable to assume that they are of no significance in the transmission of the parasites in either locality.

When obtaining the Ke and Pokoasi strains precautions were taken to collect ova from the same children in each locality. Furthermore, all possible care was taken to ensure that these children had not travelled to or lived in districts other than their own. There is good reason, therefore, to consider that the Ke strain was transmitted naturally by *B. (B.) truncatus rohlfsi* and that the Pokoasi strain was initially derived from *B. (P.) globosus*. Using these two strains of *S. haematobium* and laboratory-bred snails of *B. (B.) truncatus rohlfsi* and *B. (P.) globosus* descended from parent snails collected in the Ke Lagoon and at Pokoasi, respectively, the following experiments were conducted.

Series 1 : Exposure of B. (B.) truncatus rohlfsi and B. (P.) globosus to small numbers of miracidia of the Ke and Pokoasi strains of S. haematobium

Forty *B. (B.) truncatus rohlfsi* and forty *B. (P.) globosus* snails, between three and four months old, were taken from the laboratory breeding-tanks and divided at random into four groups, A, B, C and D, each of 20 snails. Groups A and C each contained 20 *B. (B.) truncatus rohlfsi* and groups B and D each contained 20 *B. (P.) globosus*. As shown in Table 1, groups A and B were exposed to about 200 active miracidia of the Ke strain of *S. haematobium* and groups C and D were exposed to a similar number of miracidia of the Pokoasi strain. The snails were exposed for a few hours in two Petri dishes in which were placed wooden barriers which prevented the groups of snails from intermingling, but at the same time allowed the miracidia free access and equal opportunity to develop in either *B. (B.)*

TABLE 1. RESULTS OF EXPOSURE OF *B. (B.) TRUNCATUS ROHLFSI* AND *B. (P.) GLOBOSUS* TO ABOUT 200 MIRACIDIA EACH OF THE KE AND POKOASI STRAINS OF *S. HAEMATOBium* : EXPERIMENT 1

Snails	Strain of <i>S. haematobium</i>			
	Ke strain		Pokoasi strain	
Group	A	B	C	D
Name of snail	<i>B. (B.) truncatus rohlfsi</i>	<i>B. (P.) globosus</i>	<i>B. (B.) truncatus rohlfsi</i>	<i>B. (P.) globosus</i>
Number exposed	20	20	20	20
Number infected	12	0	0	16

truncatus rohlfsi or *B. (P.) globosus*. After exposure the groups of snails were kept in separate aquaria, each offering conditions as uniform as possible, especially as regards water volume, temperature and food supply. The determination of the number of snails which subsequently became infected, by observing the emergence of cercariae, has already been described (Edwards & McCullough, 1954).

The results of this experiment (Table 1) appear to be highly significant. It can be seen that *B. (B.) truncatus rohlfsi* were markedly susceptible to the Ke strain of *S. haematobium* (12 of the 20 snails in group A became infected), but were not susceptible to the Pokoasi strain (none of the 20 snails in group C became infected). *B. (P.) globosus*, on the other hand, were strongly susceptible to the Pokoasi strain but were not susceptible to the Ke strain, as shown in Table 1, groups D and B, respectively. Furthermore, although the snails in groups B and C were apparently not susceptible to the Ke and Pokoasi strains of *S. haematobium*, respectively, they were later shown to be readily receptive when exposed to these strains in reverse order. Thus 14 *B. (P.) globosus* snails (group B) became infected following exposure to the Pokoasi strain and 15 *B. (B.) truncatus rohlfsi* (group C) were subsequently receptive to the Ke strain of *S. haematobium*. On the basis of these results it would appear that the susceptibility of the snails in each group was high, providing they were exposed to a strain of *S. haematobium* with which they were compatible. Conversely, their receptivity was low or nil when exposed to a strain with which they were incompatible. As shown in Table 2, confirmation of these findings was subsequently obtained when another experiment of similar design was conducted later.

In Tables 1 and 2 it will be noted that when *B. (B.) truncatus rohlfsi* (Table 1, group C and Table 2, group G) were exposed to the Pokoasi strain and when *B. (P.) globosus* (Table 1, group B and Table 2, group F)

TABLE 2. RESULTS OF EXPOSURE OF *B. (B.) TRUNCATUS ROHLFSI* AND *B. (P.) GLOBOSUS* TO ABOUT 200 MIRACIDIA EACH OF THE KE AND POKOASI STRAINS OF *S. HAEMATOBIMUM*: EXPERIMENT 2

Snails	Strain of <i>S. haematobium</i>			
	Ke strain		Pokoasi strain	
Group	E	F	G	H
Name of snail	<i>B. (B.) truncatus rohlfsi</i>	<i>B. (P.) globosus</i>	<i>B. (B.) truncatus rohlfsi</i>	<i>B. (P.) globosus</i>
Number exposed	16	12	12	12
Number infected	14	0	0	9

were exposed to the Ke strain of *S. haematobium*, none of the snails became infected. In view of this low degree of compatibility and the relatively few miracidia used, it was decided next to expose the snails to large numbers of miracidia in order to find out whether the Ke strain would ever be capable of developing in *B. (P.) globosus* and, likewise, whether the Pokoasi strain could infect *B. (B.) truncatus rohlfsi*. Hence the purpose of the next series of experiments was to determine whether the bulinid vectors in Ghana were quite specific to one of these strains of *S. haematobium*.

Series 2 : Exposure of B. (B.) truncatus rohlfsi and B. (P.) globosus to large numbers of miracidia of the Ke and Pokoasi strains of S. haematobium

The experimental procedure in this series was the same as that already detailed, except that the groups of snails were exposed to large numbers—2000 or more—of the miracidia of each strain. The results are shown in Tables 3 and 4.

It can be seen that *B. (B.) truncatus rohlfsi* and *B. (P.) globosus* were again markedly susceptible to the Ke and Pokoasi strains of *S. haematobium*, respectively, and indeed some of the snails died as a result of over-infection. On the other hand, only a few *B. (B.) truncatus rohlfsi* became infected after massive exposure to the Pokoasi strain (Table 3, group K and Table 4, group O). Similarly, the susceptibility of *B. (P.) globosus* was low to the Ke strain (Table 3, group J and Table 4, group N). However, the fact that there is a slight degree of receptivity in these groups indicates that the relationship between *B. (B.) truncatus rohlfsi* and the Pokoasi strain of *S. haematobium* and between *B. (P.) globosus* and the Ke strain is not absolutely specific. Nevertheless, while the vector snails can be subjected to such rigorous exposure in the laboratory, under natural conditions in the field, they will rarely, if ever, be exposed to more than a

TABLE 3. RESULTS OF EXPOSURE OF *B. (B.) TRUNCATUS ROHLFSI* AND *B. (P.) GLOBOSUS* TO MORE THAN 2000 MIRACIDIA EACH OF THE KE AND POKOASI STRAINS OF *S. HAEMATOBIMUM*: EXPERIMENT 1

Snails	Strain of <i>S. haematobium</i>			
	Ke strain		Pokoasi strain	
Group	I	J	K	L
Name of snail	<i>B. (B.) truncatus rohlfsi</i>	<i>B. (P.) globosus</i>	<i>B. (B.) truncatus rohlfsi</i>	<i>B. (P.) globosus</i>
Number exposed	15	15	15	15
Number surviving	12	15	14	10
Number infected	11	3	2	10

TABLE 4. RESULTS OF EXPOSURE OF *B. (B.) TRUNCATUS ROHLFSI* AND *B. (P.) GLOBOSUS* TO MORE THAN 2000 MIRACIDIA OF THE KE AND POKOASI STRAINS OF *S. HAEMATOBIMUM*: EXPERIMENT 2

Snails	Strain of <i>S. haematobium</i>			
	Ke strain		Pokoasi strain	
Group	M	N	O	P
Name of snail	<i>B. (B.) truncatus rohlfsi</i>	<i>B. (P.) globosus</i>	<i>B. (B.) truncatus rohlfsi</i>	<i>B. (P.) globosus</i>
Number exposed	20	20	20	20
Number surviving	13	19	20	11
Number infected	12	1	2	11

few miracidia. Consequently, in nature it is probable that *B. (B.) truncatus rohlfsi* and *B. (P.) globosus* can transmit with regularity only the strain of *S. haematobium* with which they are fully compatible.

An attempt to differentiate morphologically the Ke and Pokoasi strains of S. haematobium

In view of the apparent biological differences between the Ke and Pokoasi strains of *S. haematobium*, it was suspected that they might also differ morphologically. Measurements of 500 ova of each strain were undertaken according to the method described by Amberson & Schwarz (1953). The ova were concentrated in centrifuge tubes and were examined in the urine in which they were collected. The measurements were made with a graduated scale in the eye-piece and the length: breadth ratio of each ovum was calculated by dividing the breadth by the length (including the spine) and multiplying by a hundred. As shown in Fig. 1 and 2, the length: breadth ratios of the ova of the two strains agree very closely. In both strains the ratio of the majority of ova varied between 33 and 50, with a maximum frequency of 40. From these results it seems that measurements of ova do not offer a good basis for the morphological differentiation of the strains.

It is believed that if differences between the strains exist, they are most likely to be found in the cercarial stage of development, especially in the flame-cell formula, and the number of cephalic or penetration glands. However, although in this connexion our observations were inconclusive, it should be stressed that further investigations are called for as there appears to be considerable disagreement regarding the number of penetration glands in the cercariae of *S. haematobium* from different territories.

FIG. 1. LENGTH-BREADTH RATIOS OF 500 OVA OF *S. HAEMATOBIMUM* (KE STRAIN) RECOVERED FROM CHILDREN INFECTED AT THE KE LAGOON, WHERE *B. (B.) TRUNCATUS ROHLFSI* IS THE IMPORTANT SNAIL VECTOR

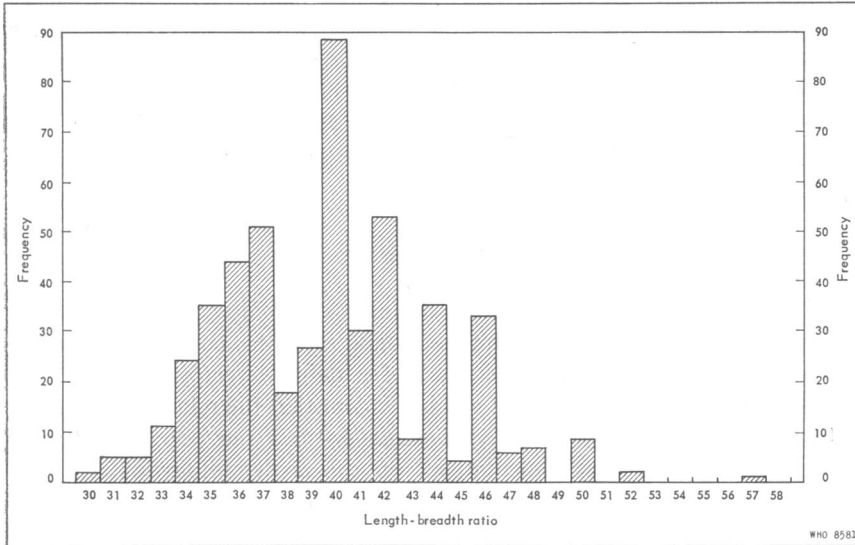
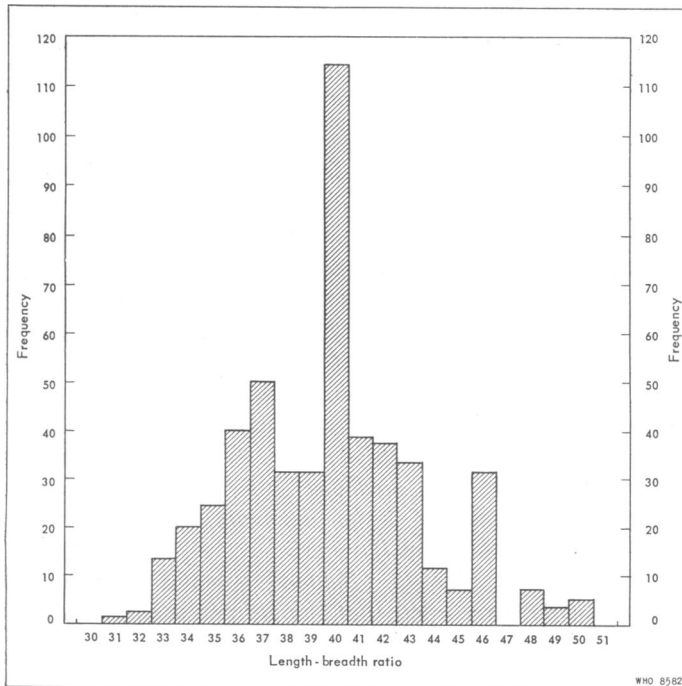


FIG. 2. LENGTH-BREADTH RATIOS OF 500 OVA OF *S. HAEMATOBIMUM* (POKOASI STRAIN) RECOVERED FROM CHILDREN INFECTED AT POKOASI, WHERE *B. (P.) GLOBOSUS* IS THE IMPORTANT SNAIL VECTOR



Most authors agree with Gordon, Davey & Peaston (1934), who worked in Sierra Leone, that there are five pairs of penetration glands, but Archibald & Marshall (1932), in a careful study of the morphology of *S. haematobium* cercariae in the Sudan, could observe only three pairs of glands. In the light of our observations on the Ke and Pokoasi strains in Ghana, these diverse findings of the above authors may well be significant, since *S. haematobium* in Sierra Leone is transmitted by *B. (P.) globosus*, whereas in the Sudan the principal vector is probably *B. (Bulinus) truncatus truncatus* (Audouin).

Discussion

Although workers have long suspected that regional strains of *S. haematobium* exist, the present experimental data seem to be the first which establish their occurrence on the basis of a definite vector-parasite compatibility or apparent lack of it. It will be evident that the relationship which exists between the strains of *S. haematobium* and the potential bulinid vectors may have a very wide impact on the epidemiology of vesicular bilharziasis not only in Ghana, but throughout Africa. Our findings at Accra may provide an explanation for some of the anomalous observations recorded by workers investigating vector-parasite relationships in other African territories. Gismann (1954) states that

"... a striking case, as regards the ability to carry *Schistosoma haematobium*, has been observed by Dr. E. G. Berry in Nigeria. In places where dense populations of both *Physopsis* and *Bulinus* occurred, natural infections was found only in the former; similarly, subsequent experiments with these same snails and Nigerian strains of *S. haematobium* gave positive results only with *Physopsis*, the natural host. When, however, an Egyptian strain of *haematobium* was used, the reverse obtained."

Considered in the light of our findings at Accra, Berry's observations in Nigeria are not surprising, especially as the natural vector in Egypt is *B. (B.) truncatus truncatus*. Moreover, when Cowper (1947) failed to infect Egyptian *B. (B.) truncatus truncatus* with a Nigerian strain of *S. haematobium* it seems probable that he was dealing with a strain highly compatible with *B. (Physopsis)* sp., as were the local strains used by Berry. Similarly, the contradictory results recently described by Le Roux (1954) and Cridland (1957) concerning the receptivity of *B. (B.) coulboisi* (Bourguignat) can probably now be resolved. Le Roux obtained positive results when he exposed this species to an Egyptian strain, while Cridland failed, as might be expected, to infect *B. (B.) coulboisi* with Uganda strains seemingly selective (as are most strains of *S. haematobium* that occur south of the Sahara) for forms of the subgenus *Physopsis*.

In the Sudan, where both *B. (Bulinus)* sp. and *B. (Physopsis)* sp. occur, Archibald (1933) recorded that the former snails were susceptible and the latter refractory to local *S. haematobium* infection. Although

satisfactory evidence is not yet available, there may be some justification for the opinion that the common strain in the Sudan is selective for *B. (Bulinus)* sp. In the course of time, however, the picture of the predominance of this strain may change, as the Sudan is widely traversed by pilgrims travelling from West and Central Africa to Mecca, who may introduce strains selective for *B. (Physopsis)* sp. from their native regions. On the other hand, it is worthy of mention that *B. (P.) africanus ovoideus* (Bourguignat) occurs in southern Sudan. In 1955, Cridland failed to infect this species in Uganda with local strains which developed readily in some other forms of the subgenus *Physopsis*. It is possible, therefore, that *B. (P.) africanus ovoideus* is quite refractory to some or all strains of *S. haematobium* and, if so, its role as a potential vector in the Sudan need not be viewed with undue suspicion. A problem of similar interest and importance to that in the Sudan seems to occur in the highlands of Ethiopia, where urinary bilharziasis is reported as endemic. In this area both the subgenera of *Bulinus* are represented, but the forms which act as the natural vector there are as yet undetermined.

In Uganda, Cridland (1955, 1957) was unable to infect forms of the subgenus *Bulinus* including *B. (B.) truncatus trigonus* (Martens), *B. (B.) transversalis* (Martens) and *B. (B.) coulboisi* with local strains of *S. haematobium*, but succeeded with both *B. (P.) nasutus* (Martens) and *B. (P.) globosus*. As already mentioned, this points to the existence in Uganda of strains especially adapted to develop in certain, though apparently not all, forms of the subgenus *Physopsis*, as infection could not be induced in either *B. (P.) africanus ovoideus* or *B. (P.) ugandae* (Mandahl-Barth). The fact that Cridland could not infect local forms of the subgenus *Bulinus* with local strains of *S. haematobium* indicates that these snails are at present unimportant as actual transmitters in Uganda. Their role as potential vectors, however, cannot be overlooked until they have been proved refractory to strains known to be compatible with forms of the subgenus *Bulinus*. At all events, there is good reason to believe that *B. (B.) coulboisi*, at least, is a potential snail host, since it is readily susceptible when exposed to a strain of *S. haematobium* with which it is compatible. Indeed, it seems probable that most bulinid snails have a latent capacity to transmit particular strains of *S. haematobium*, and this is surely a problem which, in view of its epidemiological implications, deserves our immediate attention in much of Africa.

Not all the conflicting results of other workers, however, can be resolved by assuming that there are strains of *S. haematobium* throughout Africa which are specifically adapted to snails belonging either to the subgenus *Bulinus* or to the subgenus *Physopsis*. For example, *B. (Physopsis)* sp. from South Africa were susceptible to an Egyptian strain of *S. haematobium* (Gismann, 1954). With such results in mind, it seems that the interrelationship between bulinid snails (whether potential or proven vector species) and

the parasite strains is of great complexity, depending not only on morphological and biological variation in the snail forms, but also on the existence and availability of strains of *S. haematobium* with varying degrees of compatibility. It is evident that, for a comprehensive picture to emerge, far more detailed knowledge concerning regional and inter-regional snail-schistosome relationships must be made available.

Further studies on the Ke and Pokoasi strains in *S. haematobium* in Ghana may show that they vary in pathogenicity for laboratory hosts and for man. By this approach it may be possible to reconcile the opposing views on the effect of urinary bilharziasis on health in various territories in Africa. In British West Africa, as pointed out by Blair (1956), there is general agreement that urinary bilharziasis is a mild disease, causing few symptoms and little morbidity or mortality. In Egypt, on the other hand, *S. haematobium* infection ranks as one of the most important causes of ill health and the status of the disease in that country represents the other extreme concerning the pathogenicity of the parasites. Though, in West Africa, the mildness of *S. haematobium* infection may arise from the environmental factors which at present govern the epidemiology of the disease, nevertheless it may well be more dependent on the widespread occurrence of a strain of low virulence. While the Pokoasi strain is probably ubiquitous in West Africa, the Ke strain would appear to be restricted, as assessed by the distribution of *B. (P.) globosus* and *B. (B.) truncatus rohlfsi*, respectively. If the view is taken that the strains of *S. haematobium* transmitted in West Africa by *B. (B.) truncatus rohlfsi* are closely related to Egyptian strains (bearing in mind the observations of Berry and of Cowper) it could be argued not only that the Ke strain is likely to be more pathogenic than the Pokoasi strain, but also that its more serious effect on health has been overlooked because of its rarity. It must be admitted that few data are available to support this hypothesis, but it is interesting that Gamble (1954) recently described a fatal case of vesical carcinoma associated with *S. haematobium* infection in Ghana. This condition, not uncommon in Egypt, seems to be rare in West Africa (Smith & Elms, 1934) and it may be more than a coincidence that Gamble's patient had lived for some years in a district where *B. (B.) truncatus rohlfsi* occurs.

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RÉSUMÉ

Deux souches de *Schistosoma haematobium* ont été isolées chez des enfants vivant dans les districts de Ke et de Pokoasi, près d'Accra, Ghana. Dans le district de Ke, *Bulinus (Bulinus) truncatus rohlfsi* est le vecteur le plus important, tandis qu'à Pokoasi, *Bulinus (Physopsis) globosus* est le seul hôte intermédiaire.

Les essais de transmission en laboratoire ont montré que chacun des mollusques était le vecteur pratiquement spécifique de l'une ou de l'autre souche. La souche « Ke » de *S. haematobium* se développe facilement chez *B. (B.) truncatus rohlfsi*, mais non chez *B. (P.) globosus*. Inversement, la souche « Pokoasi » se développe aisément chez *B. (P.) globosus*, mais non chez l'autre espèce.

Aucun caractère morphologique — pas même la dimension des œufs — ne permet de distinguer nettement une souche de schistosome de l'autre. Il semble que les caractères différentiels — s'ils existent — doivent être cherchés au stade cercaire.

Les observations faites au Ghana apportent des éclaircissements au problème rencontré dans d'autres régions de l'Afrique, concernant la compatibilité ou l'incompatibilité mollusque-parasite. Ces recherches, qui mettent en lumière la sensibilité ou la résistance d'espèces de bulinidés aux souches de *S. haematobium* sont une contribution intéressante à l'épidémiologie de la bilharziose urinaire, non seulement au Ghana, mais dans d'autres parties de l'Afrique. Elles sont à développer et à encourager.

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